Combining Thermal and Redox-Responses to Trigger an "Isothermal" LCST Transition.

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1. Background: Engineering Biodegradability into Responsive Materials

•Thermo-responsive polymers exhibiting a Lower Critical Solution Temperature (LCST) have been exploited for drug release[1] and hyperthermia-triggered cellular uptake.[2,3]

•For many *in vivo* applications, these materials should be degradable.[4]

•Triggering an "isothermal" LCST (changing solubility without altering temperature) may also be desirable in some cases *(e.g.* to avoid protein denaturation).[5-7]

•Here, we introduce reduction-sensitive disulphide bonds into thermo-responsive poly(*N*-isopropylacrylamide), pNIPAM, using a novel polycondensation-type procedure.

•Importantly, this material selectively degrades at intracellular (mM) glutathione (GSH) concentrations but remains stable at extracellular (μM) concentrations.[8]

•We combine this with the known molecular weight dependent LCST behaviour of pNIPAM[9] to trigger an "isothermal" transition (Fig. 1).

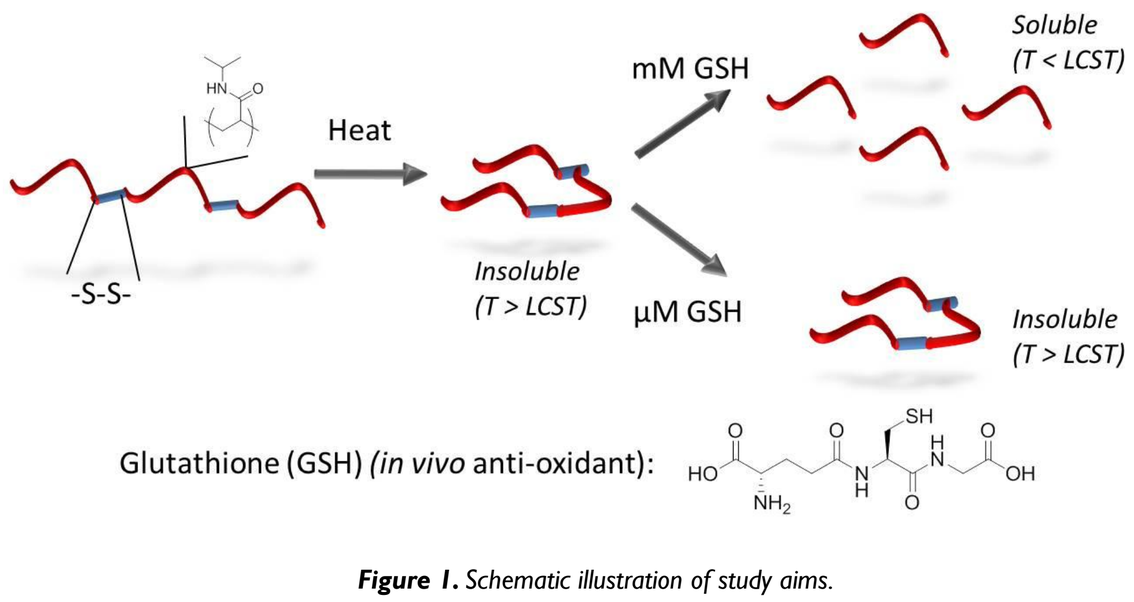

Figure 1. *Schematic illustration of study aims.*

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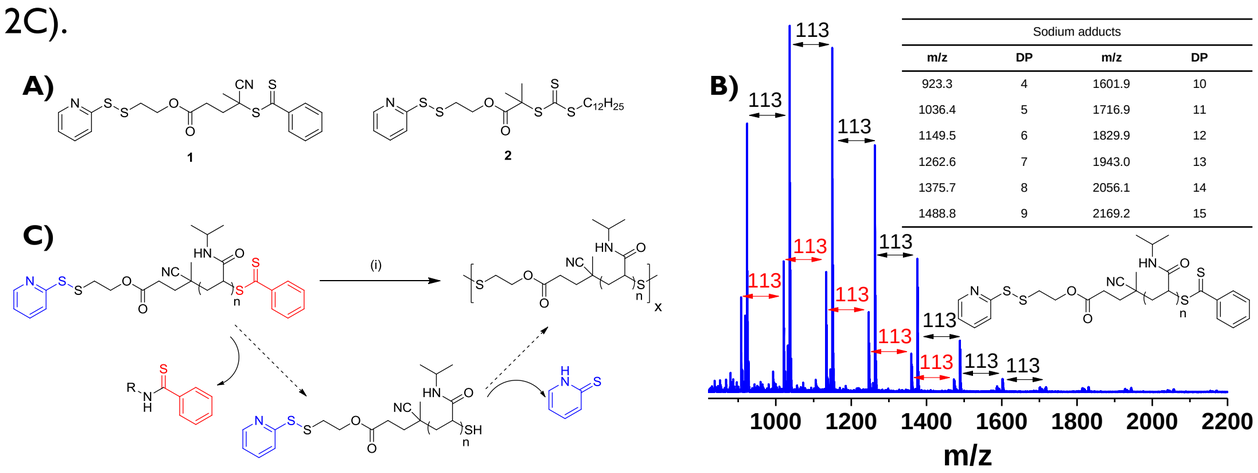
2. Preparing Disulphide-Linked Polymers

•Disulphides are formed by thiol exchange reactions with pyridyl disulphide (PDS).[10]

•Dithioester and trithiocarbonate RAFT CTAs (Fig. 2A) were used to polymerise a variety of PDS-containing (meth)acrylates and acrylamides.

•MALDI-ToF of pNIPAM prepared with CTA **1** quantified the presence of both end-groups on each chain (Fig. 2B).

•Disulphide links were introduced using a polycondensation-type procedure; aminolysis of thiocarbonyl end-group followed by *in situ* thiol-disulphide exchange (Fig. 2C).



Sodium adducts			
m/z	DP	m/z	DP
923.3	4	1601.9	10
1036.4	5	1716.9	11
1149.5	6	1829.9	12
1262.6	7	1943.0	13
1375.7	8	2056.1	14
1488.8	9	2169.2	15

Figure 2. *A) CTAs used to prepare PDS-functionalised polymers. B) MALDI-ToF spectrum of pNIPAM prepared with CTA **1**. Peaks correspond to n-NIPAM units, both end-groups and a lithium (red) or sodium (black, inset) ion. C) Polycondensation of telechelic pNIPAM: (i) Ethanolamine (1 eq.); triethylamine (2 eq.); THF; N_2; 25 °C; 24 h.*

3. Polycondensation Scope

•Disulphide linkages were successfully incorporated into pNIPAM (Fig. 3A). An increase in molecular weight from 1755 to 34000 g.mol^{-1} was observed.

•Degradability was confirmed by reduction with tributyl phosphine.

•A variety of monomers were tested (Fig. 3B). This synthetic methodology was compatible with all except non-linear poly(ethylene glycol) analogues.

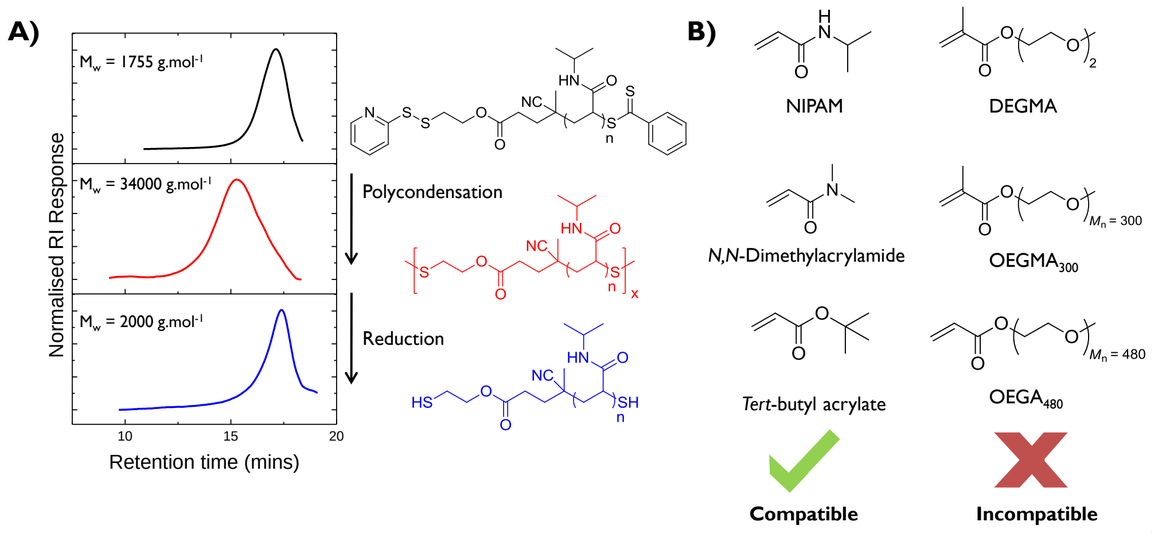


Figure 3. *A) SEC analysis demonstrating successful pNIPAM polycondensation procedure. Reduction was achieved using tributyl phosphine. B) Chemical structure of other monomers tested in this study.*

Phillips, D. J.; Gibson, M. I. *Chem. Commun.* **2012,** *48,* 1054.

4. Triggering an "Isothermal" Transition

•Disulphide-linked materials (ss-pNIPAM) from a variety of pNIPAM starting molecular weights were prepared.

•The cloud point (CP, measurable property of LCST) of pNIPAM and ss-pNIPAM (before and after incubation in mM GSH solution) was recorded (Fig. 4).

•A CP shift from 30 °C (ss-pNIPAM) to 39 °C (ss-pNIPAM, 1mM GSH) was observed.

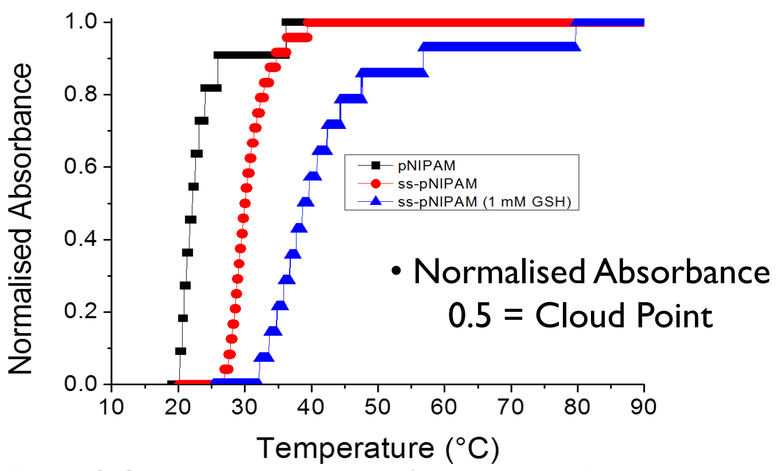


Figure 4. *Cloud point determination of pNIPAM, ss-pNIPAM and ss-pNIPAM following mM GSH incubation.*

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•This shift was used to promote an isothermal transition (Fig. 5):

• Incubation of ss-pNIPAM in water above its CP led to its precipitation;

•Treatment with mM GSH solution caused rapid re-solubilisation;

•No re-solubilisation was observed at μM GSH concentrations.

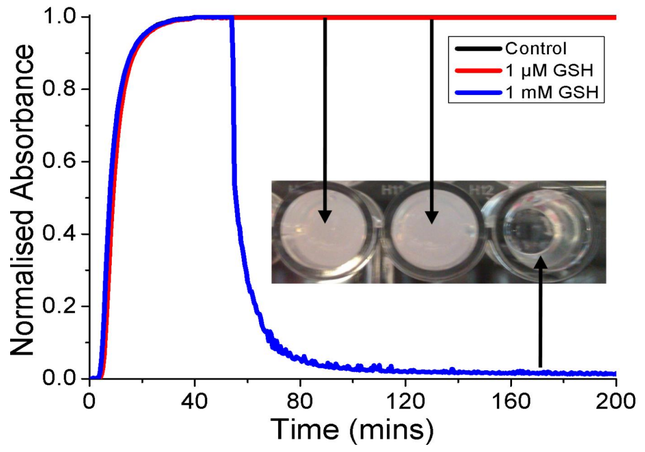

Figure 5. *Isothermal turbidimetry data for ss-pNIPAM. Temperature = 37 °C; * = GSH added. Inset: photo at end of experiment, left = control; centre = 1 μM GSH; right = 1 mM GSH.*

5. Summary

•Pyridyl disulphide-functionalised pNIPAM was prepared in a controlled manner using both dithiobenzoate and trithiocarbonate chain transfer agents.

•Disulphides were incorporated into the polymeric structure *via* a polycondensation-type reaction between a terminal thiol and pyridyl disulphide group.

•Selective degradability of disulphide linkages to intracellular glutathione concentration was demonstrated.

•This was combined with the known molecular weight dependent LCST of pNIPAM to produce an isothermal transition.

•Future work will use these materials to trigger mammalian cellular uptake.

6. References

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7. Acknowledgements

MIG Group

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